Clearance of Inhaled Ceramic Fibers From Rat Lungs

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Deposition, clearance, retention, and durability of inhaled particles in lungs are important factors for induction of pulmonary fibrosis or lung cancer. To study the deposition and clearance of aluminium silicate ceramic fibers from the lung, male Wistar rats were exposed to ceramic fibers, with a mass median aerodynamic diameter (MMAD) of 3.7 µm, for 6 hr/day, 5 days/week for 2 weeks. The average exposure concentration was 27.2 mg/m^3 (SD 9.0). The rats were killed at 1 day, 1 month, 3 months, and 6 months after the end of exposure, and the fiber numbers and dimensions were measured with a scanning electron microscope. No significant difference in length of residual ceramic fibers in the lungs was found among the groups. The geometric mean diameter and number of ceramic fibers, however, decreased according to the clearance period. These findings suggest that the fibers were dissolved at their surface. — Environ Health Perspect 102(Suppl 5):169–171 (1994)

Key words: inhalation, ceramic fiber, clearance, solubility

Introduction

Occupational exposure to various types of asbestos causes lung fibrosis (asbestosis), bronchial cancer, and pleural and peritoneal mesotheliomas. The use of asbestos fibers has decreased greatly in recent years and dust controls have improved. On the other hand, because of asbestos substitutes and new industrial materials, the global production of man-made mineral fibers (MMMF) has increased (*1*). The safety of MMMF has been questioned because of their similarity in shape and chemical composition to asbestos. Physical dimension, clearance, and durability of fibers in lungs were reported as important factors in predicting the biological effect of inhaled fibers. A number of studies on the clearance and solubility of inhaled MMMF have been reported (*2–7*). In this study, we exposed rats to ceramic fibers by inhalation and determined the changes of numbers, lengths, and diameters of ceramic fibers retained in rat lungs after various clearance periods after the end of exposure.

Materials and Methods

Test Substances

Ceramic fibers (aluminium silicate refractory fibers with a nominal diameter of 2–3 µm) used in this study were made by a Japanese manufacturer. The chemical composition of the fiber was SiO_2, 54% and Al_2O_3, 46%. To obtain the respirable fiber particles that have mass median aerodynamic diameter (MMAD) of less than 5 µm, bulk ceramic fibers were disintegrated three times with an ultracentrifugal mill (Retch Co., Germany) at a speed of 15,000 rpm.

Exposure System and Exposure Chamber

Details of our system and apparatus have been reported previously (*1,6–8*).

Concentration and Size Distribution in the Exposure Chamber

The mass concentration of ceramic fibers was measured gravimetrically at daily intervals by the isokinetic suction of air in the chamber through a glass filter. The average concentration was 27.2 mg/m^3 (standard deviation [SD] 9.0). The size distribution of the aerosol in the exposure chamber was determined with an Andersen cascade impactor (Model AN-200, Sibata Scientific Technology Ltd., Japan). The MMAD of the ceramic fibers in the chamber was 3.7 µm with geometric standard deviation (GSD) of 2.2.

Rats

Male Wistar rats, 9 weeks old at the start of exposure, were used. The rats were exposed to ceramic fibers for 6 hr/day, 5 days/week, for 2 weeks. They were then killed at 1 day, 1 month, 3 months, and 6 months after the end of the exposure.

Determination of Ceramic Fibers in Rat Lungs

Ceramic fibers were extracted from rat lungs by low temperature ashing (*6*). Number, length, and diameter of fibers were determined with a scanning electron microscope (SEM: Hitachi S-700) according to the reference methods for measuring airborne MMMF proposed by the World Health Organization (WHO) (*9*). The filters were cut into approximately 7 mm^2 after drying, then put onto the SEM stubs. They were sputter-coated with 7 nm of platinum and examined by SEM. Photomicrographs of the filters were taken randomly at a fixed magnification (× 2000). If any of the fibers had only one end within the field of view, the second photomicrograph was recorded at lower magnification (× 1000) and centered on the original field so that the fiber length could be assessed. The films were printed on 25 × 30 cm photographic papers. The bulk of refractory ceramic fiber included large nonfibrous particles of ceramic. When this bulk of ceramic fiber was disintegrated, the product consisted of fibrous and nonfibrous particles. In this study, only particles that had an aspect ratio ≥3:1 were counted and measured as ceramic fibers. In counting the number of ceramic fibers on the photographs, those with two ends in the field were scored 1 and those with one end in the field, 0.5. If both ends were out of the field, the fiber was not counted. The numbers retained in each rat were calculated on the basis of the WHO reference method (*9*). The size of fibers was measured by using the backlight digi-

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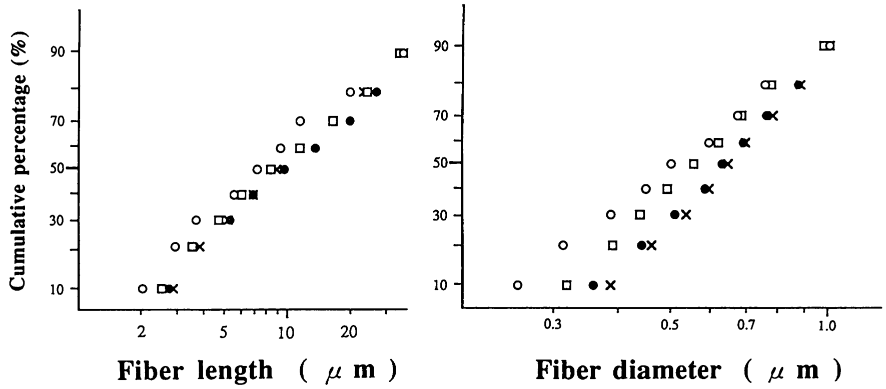

Figure 1. Distribution of fiber length and fiber diameter after 1 day (3), 1 month (d), 3 months (h), and 6 months (s) clearance time.

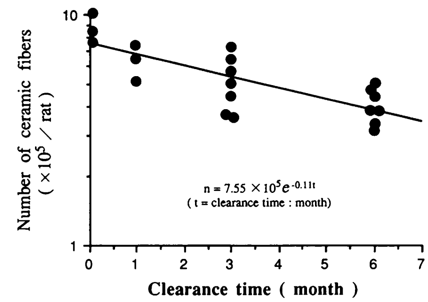

Figure 2. Changes in numbers of ceramic fibers retained in rat lungs. The regression line and the equation were obtained by the least square method.

tizer (KD3030L GRAPHTEC, Japan). The size distributions of inhaled ceramic fibers were calculated for all rats at each clearance period.

Results

Fiber Dimensions in Rat Lungs

The size distributions of the recovered fibers were log normal as illustrated by the cumulative distributions shown in Figure 1. The geometric mean length (GML) and diameter (GMD) of the fibers were assessed from them. There was no significant difference in the GML until three months after the end of exposure. The GMD decreased, however, as the duration of the clearance period lengthened.

Numbers of Ceramic Fibers in Rat Lungs

Figure 2 shows the changes in numbers of fibers in the lungs with time. The numbers of ceramic fibers retained decreased as the clearance period lengthened. The results are summarized in Table 1.

Discussion

This report describes a series of experiments designed to study the clearance of inhaled MMMF from rat lungs, and shows that the number and diameter of inhaled ceramic fibers decreased in proportion to the length of the clearance period. The decrease in number of ceramic fibers retained in rat lungs was statistically significant in contrast to a previous study in which a tendency of decrease of number observed was not statistically significant, since only three to four rats were used instead of three to seven in the present study. However, a linear decrease in the diameter of ceramic fibers with the lengthening of clearance period was observed in both studies, suggesting that dissolution of ceramic fibers occurred at their surfaces.

Previous reports (*2–6*) also have described the dissolution of MMMF in animal lungs.

Bernstein et al. (*2*) examined the clearance of radioactive glass fibers that had known size distributions (GMD, 1.5 µm, length of either 5 or 60 µm) from Fischer 344 rats. There was little difference in clearance time between long fibers (half-life, 35.0 days) and short fibers (half-life, 38.5 days) at 19 weeks after instillation, but the short fibers were apparently successfully phagocytized by alveolar macrophages and cleared to the lymph nodes, whereas the long fibers were not. The long fibers also produced a granulomatous response in giant cells.

When Morgan et al. (*3*) administered to rats fibers 5, 10, 30 and 60 µm in length and 1.5 µm in diameter, the clearances after 12 months determined by fiber count were 90% for fibers 5 µm long, 80% for fibers 10 µm long; fibers 30 and 60 µm long were not cleared to any significant extent.

However, the dissolution of the fibers, as shown by the decrease in the count median diameters (CMD) was 28% after 12 months for the fibers 10 µm long; for those 30 µm and 60 µm in length, the CMD was less than half that of the original after 18 months. Therefore, longer fibers appeared to be more soluble than shorter ones.

Holmes et al. (*4*) administered sized glass fibers to hamsters by intratracheal instillation. The count median length (CML) for 10 µm fibers increased over the first three months, whereas those of the longer fibers (30, 60, and 100 µm) remained relatively constant. They also showed that the CMDs decreased almost linearly, but at different rates, for short fibers (10 µm) and for long fibers (30 and 60 µm). They also compared the rate of decrease of CMDs in rat lungs and hamster lungs, and found that glass fibers dissolved much more rapidly in the hamster. They concluded that the difference in these rates of dissolution was due to intrinsic differences in physiological pH of lung or of pulmonary cells.

Leineweber (*5*) has shown that the solubility of MMMF *in vitro* varied over a wide range and depended on the chemical composition of the fibers, and that

Table 1. Total number, diameter and length of inhaled ceramic fibers in rat lungs.

Clearance time	Number of tested rats	Number of CF, 10^5/rat	GMD[a] (GSD)[b], µm	GML[c] (GSD), µm
1 day	3	8.7 ± 1.3	0.65 (1.4)	9.0 (2.9)
1 month	3	6.3 ± 1.1	0.63 (1.5)	9.5 (3.2)
3 months	7	5.2 ± 1.4	0.56[d] (1.5)	8.6 (3.1)
6 months	7	4.0 ± 0.7[d]	0.50[d] (1.7)	7.1 (3.0)

[a]Geometric mean diameter [b]Geometric standard deviation [c]Geometric mean length [d]Significantly different ($p < 0.01$), compared with the values of one day after the exposure. The values of the number of ceramic fibers retained in each rat were means ±SD. Statistical comparisons of the change of fiber number, fiber length, and diameter among each clearance period were analyzed by a two sample *t*-test. The data of length and diameter were compared after log transformation.

the rates of dissolution observed in the above studies—which were much greater than in the present study—were due to the differences in chemical composition of ceramic and glass fibers. These findings suggest that the solubility of inhaled MMMF is a very important factor for clearance from the lung.

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